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The Influence of Moisture on Variation in the Eggs and Hatchlings of *Anolis c. carolinensis* Voigt

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The eggs of reptilian species with leathery shells are known to change in size, shape, volume and weight throughout the incubation period. Relatively few studies have presented a detailed and adequate picture of these changes. Many fragmentary reports, as well as more detailed studies, have been based on eggs either laid in captivity or discovered in natural nests, and then incubated under laboratory conditions. Few workers have compared the results of incubation under laboratory conditions with those obtained under field conditions. No one has considered the effect of bringing eggs, partially incubated in the field, into the laboratory. The evidence brought forth in this paper indicates that measurements of field incubated eggs and hatchlings may vary significantly from those of laboratory incubated eggs and hatchlings.

As a part of investigation of anoline populations in Louisiana, studies of the gross changes in eggs during development were made. The initial study was conducted in 1953 in the New Orleans region ; experimental work was carried out in 1957 at Monroe, Louisiana.

METHODS

For the initial study eggs were obtained from the natural environment and from females in an artificial environment. Gravid females were placed in cages containing a substrate of Spanish moss. The substrate was inspected for eggs (hereafter referred to as laboratory eggs) twice daily. Eggs were also collected in the field (field eggs) and brought into the laboratory for completion of incubation.

Both types of eggs were treated in the following manner. A number was penciled on each egg; linear measurements were made with Vernier calipers ; weights were taken on a pharmaceutical balance. The eggs were placed in an incubator consisting of a covered pint mason jar con-

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taining moist paper toweling. The eggs were incubated to hatching at room temperatures which varied from 72 to 95° F. The toweling was changed at least once a week. Moisture on the inner walls of the jar indicated a saturated atmosphere. Additional measurements were taken at weekly intervals until the approach of hatching ; at this time daily measurements were made, thus insuring the acquisition of data within 24 hours of hatching.

The experimental study in 1957, utilized much of the above routine. Wire shelves were placed approximately two inches above the bottom of the incubator. Approximately one inch of water was put into each incubator. Cotton tapes served as wicks from the water to the shelf. Eggs obtained from gravid females were divided into three groups. One group was exposed to a regimen of moisture for two days, dryness for one ; another to the reverse conditions ; and a third served as a control with

TABLE 1. Measurements of anole eggs at various stages of development

Period	Mean	SE_M	SD	N	Range	t
			MAXIMUM LENGTH (in mm.)			
Deposition	10.13	0.05	0.46	75	9.4 -11.3	
7-8 days	9.96	0.08	0.44	33	9.0 -11.0	
20-21 days	10.34	0.08	0.44	27	9.6 -11.2	
Hatching[1]						
Lab[2]	10.25	0.10	0.45	20	9.7 -11.4	
Hatching						7.24
Field[3]	11.93	0.21	0.86	17	10.6 -13.6	
			MAXIMUM WIDTH (in mm.)			
Deposition	6.07	0.04	0.37	75	5.3 - 7.1	
7-8 days	7.32	0.07	0.41	33	6.5 - 8.4	
20-21 days	8.61	0.09	0.46	27	7.4 - 9.4	
Hatching						
Lab	8.09	0.14	0.64	20	6.8 - 9.2	
Hatching						6.63
Field	9.71	0.20	0.84	17	8.2 -11.0	
			WEIGHT (in gms.)			
Deposition	0.22	0.03	0.02	54	0.17- 0.29	
7-8 days	0.32	0.01	0.03	33	0.27- 0.41	
20-21 days	0.44	0.01	0.06	27	0.32- 0.54	
Hatching						
Lab	0.38	0.01	0.07	21	0.30- 0.54	
Hatching						5.82
Field	0.62	0.04	0.15	15	0.44- 0.92	

[1] Measurements within 24 hours of hatching

[2] Eggs incubated under laboratory conditions for entire developmental period

[3] Eggs obtained in the field, incubated for varying lengths of time in the laboratory

incubation conditions similar to those of the initial study. The program for the two experimental groups was modified at the end of two weeks. The number of days of moisture was increased to four, alternating with one day of dryness for the first group, and for the second group, 1 day of moisture and 4 days of dryness. The incubators were maintained at temperatures varying from 70° to 78°F.

Statistical significance was ascertained by use of the Student "t" test as advocated by Hubbs and Perlmutter (1942) .

RESULTS

At deposition the anole egg is ovoid in shape (Table 1) . The embryo is in a relatively advanced state of development, at least beyond the early somite stage (Hamlett, 1952) . *Anolis* eggs increase in length and width as well as weight until the ninth or tenth day prior to hatching ; the weight doubles and the egg becomes spherical in shape (Table 1) . Measurements within 24 hours of hatching indicate a decrease in weight and a return to the ovoid shape. The weight decrease is accompanied by the appearance (and subsequent evaporation) of small beads of fluid on the surface of the egg. The beads are present regardless of the incubation conditions and indicate that the incubation period is near termination. Since changes in weight represent changes in the moisture content of the egg (Cunningham and Hurwitz, 1936) , it is assumed that the beads are primarily water. Perhaps the loss of fluid serves as a stimulus to initiate the movements leading to hatching —a paradoxical situation in a mechanism designed to conserve moisture.

The size of the egg within 24 hours of hatching varies according to the conditions to which it has been exposed throughout the incubation period. Hamlett (1952) incubated *Anolis* eggs in soil and leaf mold "at laboratory temperatures" and reported eggs 17 x 12 mm. in size at time of hatching. Twenty laboratory eggs averaged 10.3 x 8.1 mm. in maximum length and width, respectively, and had a mean weight of 0.38 gms. Seventeen field eggs maintained in the laboratory under similar incubation conditions for varying periods of time (1 to 23 days) before hatching, had an average maximum length and width of 11.9 x 9.7 mm. and a mean weight of 0.62 gm. The differences between the field and laboratory eggs are statistically significant (Table 1) . Indeed, if one is accustomed to examining eggs of *Anolis* obtained only from gravid females, the initial inspection of eggs incubated under natural conditions can be startling (note the extremes in Table 1) . The field eggs were heterogeneous with regard to age. If the field sample, measured just

prior to hatching, consisted of eggs incubated under field conditions for all but one or two days of the incubation period, the differences between the field and laboratory eggs would be presumably more distinct than is indicated above.

TABLE 2. Snout-vent length and weight of hatchling anoles from laboratory and field eggs

Source	Mean	SE_M	SD	N	Range	t
Snout-vent length (in mm.)						
Lab	20.40	0.13	0.88	44	18.0-21.9	6.97
Field	22.36	0.25	1.31	28	19.4-24.4	
Weight (in gms.)						
Lab	0.24	0.01	0.05	40	0.170-0.340	3.07
Field	0.28	0.01	0.04	30	0.207-0.386	

The mean snout-vent length and weight of hatchlings derived from the two types of eggs are, as expected, significantly different (Table 2) .

Since both field and laboratory eggs were drawn from the same population, and the field eggs were incubated in the same manner as the laboratory eggs (in many cases both types in the same incubator) , the differences obtained must have developed while the field eggs were exposed to natural conditions. Of the two environmental factors most often thought to be of primary importance during development, moisture varied in the field, but was constant in the incubators ; temperatures varied in both field and laboratory. The role of light and/or irradiation, which differed considerably between the field and laboratory, is not known.

TABLE 3. Snout-vent length and weight of hatchling anoles incubated under varying moisture conditions

Conditions	Mean	SE_M	SD	N	Range	t
Snout-vent length (in mm.)						
Dry intermittent	22.00	0.46	1.31	8	21.0-24.0	4.14
Wet intermittent	24.75	0.48	0.96	4	24.0-26.0	
Weight (in gms.)						
Dry intermittent	0.27	0.02	0.05	10	0.21-0.35	4.64
Wet intermittent	0.40	0.02	0.05	4	0.33-0.43	
Continuous moist	0.24	0.01	0.02	3	0.22-0.25	

As indicated in the section on methods, an experiment to determine the role of moisture in relation to egg and hatchling size was conducted. Although a smaller number of eggs was utilized, the results (Table 3) are suggestive of the influence of intermittent moisture in determining hatchling size. Hatchlings obtained from eggs maintained on wet wicks for more than 70% of the incubation period are significantly larger in snout-vent length and weight than those from eggs maintained in a saturated atmosphere or off wicks for at least 70% of the incubation period.

Comparison of snout-vent length of laboratory and field hatchlings (Table 2) with those of the experimental animals (Table 3) cannot be made. While both groups of eggs were collected from populations in Louisiana, the former are from the New Orleans region, while the latter are from the Monroe area. The differences between the two tables may reflect geographic variation, the extent of which, for reptilian eggs and hatchlings, is unknown at the present time.

DISCUSSION

Blanchard (1926) found that the eggs of *Diadophis punctatus edwardsii* increased in size "until a week or so before hatching." At this time the eggs either ceased increasing or actually decreased in size. Lynn and von Brand (1945) reported an increase in weight for eggs of *Chelydra, Chrysemys* and *Terrapene,* but mentioned no decrease in weight prior to hatching. Crenshaw (1955) noted an increase in length and width of *Sceloporus u. undulatus* eggs during development. A decrease in weight of the eggs of *Sceloporus undulatus* was recorded by Cunningham and Hurwitz (1936) within a two week period prior to hatching. The data presented for *Anolis* indicate that development is accompanied by a pronounced increase in linear and weight measurements, followed by a decrease during the week preceding hatching. No correlation can be drawn for the various reptile groups, since it is difficult to interpret the vast majority of reports on reptile eggs in the literature. Either the relative age at the time of measurement is not given, or the conditions of incubation are withheld.

The eggs of *Anolis,* at least in the New Orleans region, are laid either beneath superficial surface debris, or quite openly on the ground. In areas of heavy population densities, it is not unusual to find a colonial egg-laying site, with viable eggs, and shells of hatched and parasitized eggs, lying at random on the soil surface. Such eggs are exposed to a cycle of daily rain and drying characteristic of the Gulf Coast region

The alternate wetting and drying is believed to be the primary factor accounting for the difference in size between eggs incubated solely in the laboratory and eggs in which part of the incubation occurs under natural conditions. Clark (1953) observed that eggs of *Elaphe emoryi,* which were partially desiccated, absorbed water and attained a larger size than eggs maintained under conditions of continuous, constant water supply.

On the basis of unpublished data, the differences in body length found in hatchlings obtained from the two types of eggs do not appear to be sufficient to produce two separate size groups at the end of the growing season.

The ability of the reptile egg to take up greater amounts of moisture following dehydration is obviously of survival value, especially where the egg is given little or no protection in a nest.

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